occuring mammalian thrombopoietin, wherein the DNA segment comprises a region that is at least 80% identical to the region between nucleotides number 237 and 722 of SEQ ID NO:1, and wherein said thrombopoietin stimulates MPL-dependent cell proliferation; and

a transcription terminator.

22. (amended) An expression vector according to claim 20 wherein said DNA segment comprises nucleotide 237 to nucleotide 722 of SEQ ID NO: 1 [or nucleotide 64 to nucleotide 525 of SEQ ID NO: 18].

Please cancel claims 12, 14, 15, 16, 17, 18, 19, 21, 25, 26, and 37 without prejudice.

REMARKS

The undersigned representative thanks the Examiner for her courtesy in conducting an interview on August 1, 1995.

Entry of the above amendments is earnestly requested. With these amendments claims 10, 11, 13, 20, 22-24, 27, 28, 32 and 33 are now in this case. Claims 12, 14-19, 21, 25, 26 and 37 have been canceled. Claims 10, 11, 13, 20 and 22 have been amended. Applicants reserve the right to prosecute the canceled subject matter in one or more continuing applications.

The above amendments have been made to accelerate prosecution on certain preferred embodiments of Applicants' invention. In addition, the specification has been amended to correct a clerical error. No new matter has been added.

Claims 10 and 20 have been amended to recite that the polynucleotide or DNA segment, respectively, encodes naturally occuring mammalian thrombopoietin, wherein the polynucleotide or DNA segment comprises a region that is at least 80% identical to the region between nucleotides number

237 and 722 of SEQ ID NO:1, and wherein said thrombopoietin stimulates MPL-dependent cell proliferation. Claim 11 has been amended to recite "nucleotide 722". Claim 13 has been amended to recite "amino acid residue 206". Support for these limitations is found within Applicants' specification, such as at page 5.

Applicants have disclosed representative clones of naturally occuring mouse and human thrombopoietins, including their sequences, and have deposited representative clones with the American Type Culture Collection. Applicants have further disclosed methods for using these clones and sequence information obtained therefrom in cloning other mammalian thrombopoietin-encoding polynucleotides. See, for example, Applicants' specification at pages 11-13, and Example IX. Briefly, one prepares a cDNA library from mRNA obtained from a tissue shown to contain mRNA encoding TPO, such as lung, liver, heart, skeletal muscle, or kidney. These tissues are readily available in the art. The library is then probed with one of the disclosed DNAs or a fragment thereof, or with one or more small probes based on the disclosed sequences. Such methods are routine in the art, and are disclosed in detail in well-known laboratory manuals, including Ausubel et al., eds., Current Protocols in Molecular Biology, John Wiley and Sons, NY 1987; and Sambrook et al., eds., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, 1989 (cited on page 11 of Applicants' specification). Applicants have provided working examples in which human cDNA and genomic clones were obtained through the use of sequence information from a mouse clone and conventional cloning techniques.

Polynucleotides encoding TPO are recognized by their degree of sequence identity to known TPO clones and by the biological activity of the encoded protein. The claimed polynucleotides are at least 80% identical in sequence to

the region between nucleotides number 237 and 722 of SEQ ID NO:1. As disclosed on page 18 of the specification, thrombopoietin has a two-domain structure, wherein the amino-terminal region of thrombopoietin forms a cytokine-like or erythropoietin-like domain. See also Table 3 on page 21 of the specification. This cytokine-like domain, which resides within the region bounded by residues 45-206 of SEQ ID NO:2 (encoded by nucleotides 237 to 722 of SEQ ID NO:1), is highly conserved between species. For the Examiner's convenience, an alignment of the mouse and human TPO cDNA sequences (attached hereto as Appendix A) is included with this amendment. This alignment was prepared using analytical methods that are standard in the art.

Determination of polynucleotide sequences is routine in the art. Early, manual methods of sequencing have been supplanted by automated techniques that have made possible the sequencing of a large part of the human genome in a remarkably short time. Thus, hybridizing clones identified by methods taught in Applicants' disclosure can be readily sequenced, and the sequences compared to SEQ ID NO:1. Degree of sequence identity is readily determined by alignment and analysis using well known computer programs.

Stimulation of MPL-dependent cell proliferation can be determined as disclosed in Applicants' specification, such as at page 9 and in the Examples (e.g. at page 67).

In view of Applicants' disclosure and the relative skill and knowledge of those in the art, the ordinarily skilled practitioner could readily utilize the sequence information provided by Applicants to clone additional polynucleotides encoding naturally occuring mammalian thrombopoietins having the recited degree of sequence identity and the recited biological activity.

Applicants believe that the amended claims are allowable, and notice to that effect is respectfully requested. If for any reason the Examiner feels that a telephone conference would expedite prosecution of the

application, the Examiner is invited to telephone the undersigned at (206) 442-6673.

Respectfully Submitted,
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Enclosures:
Appendix A (sequence alignment)

Comparison of:
(A) zg-htpo-cod.fseq >zg-htpo124 - 1062 nt
(B) zg-mtpo-cod.fseq - 1080 nt
using DNA matrix

81.1% identity in 1074 nt overlap; init: 2039, opt: 2811

APPENDIX A

```
            10        20        30        40        50        60
zg-htp ATGGAGCTGACTGAATTGCTCCTCGTGGTCATGCTTCTCCTAACTGCAAGGCTAACGCTG
       X:::::::::::::: :::::::: : :: :::::::::   :   ::::: ::::: :::
>zg-mt ATGGAGCTGACTGATTTGCTCCTGGCGGCCATGCTTCTTGCAGTGGCAAGACTAACTCTG
     10        20        30        40        50        60

            70        80        90       100       110       120
zg-htp TCCAGCCCGGCTCCTCCTGCTTGTGACCTCCGAGTCCTCAGTAAACTGCTCCGTGACTCC
       :::::::: :   ::::::: ::::::: : :: :::: : :::::::::: :::::::::
>zg-mt TCCAGCCCCGTAGCTCCTGCCTGTGACCCCAGACTCCTAAATAAACTGCTGCGTGACTCC
     70        80        90       100       110       120

           130       140       150       160       170       180
zg-htp CATGTCCTTCACAGCAGACTGAGCCAGTGCCCAGAGGTTCACCCTTTGCCTACACCTGTC
       ::  :::::::::::: :::::::: ::::: :: :: ::  :::::::: :::  :::::
>zg-mt CACCTCCTTCACAGCCGACTGAGTCAGTGTCCCGACGTCGACCCTTTGTCTATCCCTGTT
     130       140       150       160       170       180

           190       200       210       220       230       240
zg-htp CTGCTGCCTGCTGTGGACTTTAGCTTGGGAGAATGGAAAACCCAGATGGAGGAGACCAAG
       :::::::::::::::::::::::: :::::::::::::::::::: :::  ::: ::::
>zg-mt CTGCTGCCTGCTGTGGACTTTAGCCTGGGAGAATGGAAAACCCAGACGGAACAGAGCAAG
     190       200       210       220       230       240

           250       260       270       280       290       300
zg-htp GCACAGGACATTCTGGGAGCAGTGACCCTTCTGCTGGAGGGAGTGATGGCAGCACGGGGA
       ::::::::::::::: :: :::::: :::::::: :::::::::::::::::::::::: :::
>zg-mt GCACAGGACATTCTAGGGGCAGTGTCCCTTCTACTGGAGGGAGTGATGGCAGCACGAGGA
     250       260       270       280       290       300

           310       320       330       340       350       360
zg-htp CAACTGGGACCCACTTGCCTCTCATCCCTCCTGGGGCAGCTTTCTGGACAGGTCCGTCTC
       ::  ::: :::: : :::::::::::::::::::: :::::::::: ::::: :: :::
>zg-mt CAGTTGGAACCCTCCTGCCTCTCATCCCTCCTGGGACAGCTTTCTGGGCAGGTTCGCCTC
     310       320       330       340       350       360

           370       380       390       400       410       420
zg-htp CTCCTTGGGGCCCTGCAGAGCCTCCTTGGTACCCAGCTTCCTCCACAGGGCAGGACCACA
       ::: : :::::::::::: :::::::: :: :::::::::::: :::::::::::::::::
>zg-mt CTCTTGGGGGCCCTGCAGGGCCTCCTAGGAACCCAGCTTCCTCTACAGGGCAGGACCACA
     370       380       390       400       410       420

           430       440       450       460       470       480
zg-htp GCTCACAAGGATCCCAATGCCATCTTCCTGAGCTTCCAACACCTGCTCCGAGGAAAGGTG
       :::::::::: ::::::::: ::::: ::::::: ::::: ::::: :: :::::::::
>zg-mt GCTCACAAGGACCCCAATGCCCTCTTCTTGAGCTTGCAACAACTGCTTCGGGGAAAGGTG
     430       440       450       460       470       480

           490       500       510       520       530       540
zg-htp CGTTTCCTGATGCTTGTAGGAGGGTCCACCCTCTGCGTCAGGCGGGCCCCACCCACCACA
       :: :::::: : :: :::: ::: ::::::::::: ::::: ::: :::  :: ::::::
>zg-mt CGCTTCCTGCTTCTGGTAGAAGGTCCCACCCTCTGTGTCAGACGGACCCTGCCAACCACA
     490       500       510       520       530       540

           550       560       570       580       590       600
zg-htp GCTGTCCCCAGCAGAACCTCTCTAGTCCTCACACTGAACGAGCTCCCAAACAGGACTTCT
       :::::::: ::::: :: :::: : :::::::::: ::: :: ::::::::::::::::::
>zg-mt GCTGTCCCAAGCAGTACTTCTCAACTCCTCACACTAAACAAGTTCCCAAACAGGACTTCT
     550       560       570       580       590       600

           610       620       630       640       650       660
zg-htp GGATTGTTGGAGACAAACTTCACTGCCTCAGCCAGAACTACTGGCTCTGGGCTTCTGAAG
       :::::::::::::: ::::::: :: : :::::::::::: ::::: :::: ::::::::
>zg-mt GGATTGTTGGAGACGAACTTCAGTGTCACAGCCAGAACTGCTGGCCCTGGACTTCTGAGC
     610       620       630       640       650       660

           670       680       690       700       710
zg-htp TGGCAGCAGGGATTCAGAGCCAAGAT---TCCTGGTCTGCTGAACCAAACCTCCAGGTCC
        :::  :::::::::::::: ::::::   V::^:::: ::: :: ::::::::::::::::
>zg-mt AGGCTTCAGGGATTCAGAGTCAAGATTACTCCTGGTCAGCTAAATCAAACCTCCAGGTCC
     670       680       690       700       710       720
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